Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at http://about.jstor.org/participate-jstor/individuals/early-journal-content.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

THE INFLUENCE OF BRILLIANT GREEN ON THE DIPHTHERIA BACILLUS

J. A. KOLMER;. S. S. WOODY, AND E. M. YAGLE

From the Philadelphia Hospital for Contagious Diseases and the Dermatological Research Laboratories, Philadelphia.

Owing to the widespread distribution and prevalence of diphtheria and the tendency for the bacilli to persist for weeks and months in the tonsils and other tissues of the upper respiratory tract after an attack of the disease, any method promising a practical means for the disinfection of carriers is gratefully but somewhat skeptically received by those particularly interested in this problem.

Our interest in the use of solutions of brilliant green for the disinfection of diphtheria bacilli carriers was stimulated by Browning,[1] who states that the unpublished investigations of Mackie indicate that spraying 1:500-1:250 solutions of brilliant green over the fauces, tonsils and pharynx three times daily for a week, succeeded in eliminating diphtheria bacilli from the throats of chronic carriers whose infections had resisted various other forms of local treatment over periods of many months. Krumwiede and Pratt,[2] during the course of an extensive investigation on the influence of 40 dyes on 30 different pathogenic bacteria, found that dilutions of Grübler's and Bayer's brilliant green in agar as high as 1:500,000 restrained the growth of diphtheria and pseudodiphtheria (diphtheroid) bacilli, although the results were somewhat irregular, owing to the tendency for cultures to produce flakes and the failure of isolated bacilli to grow in plates. As shown by Krumwiede and his associates, brilliant green behaves in a somewhat similar manner as gentian violet toward gram-positive and gram-negative micro-organisms, being more highly bactericidal and antiseptic for the former. The dye has been used with considerable success in the isolation of typhoid bacilli from feces because it exerts an antiseptic influence on many bacteria of the fecal flora in dilutions that have no influence on the typhoid bacillus.

Received for publication Oct. 27, 1919.

---

[1] Applied Bacteriology, 1918, p. 87.

[2] Jour. Exper. Med., 1914, 19, p. 501.

We have studied the bactericidal activity of brilliant green in different culture mediums on one strain of the diphtheria bacillus (Park-Williams No. 8), with comparative tests employing B. typhosus, B. coli (communis) and Staph. aureus; also the influence of a 1:250 water solution of the dye applied three times daily to the fauces, noses and ears of a number of carriers of virulent and nonvirulent bacilli. The results are summarized in this paper.

THE BACTERICIDAL ACTIVITY OF BRILLIANT GREEN IN VITRO

Grübler's and Bayer's brilliant green were employed; the former occurred in greenish gold crystals readily soluble in water and permitting the preparation of solutions as concentrated as 1:10, the solution having a bright green color. Bayer's dye was of a bluish green color and not quite as soluble in water, 5% solution being near the limit of perfect solubility. The two dyes, however, were about equal in bactericidal activity; differences were slight and usually within the range of experimental error in disinfection tests.

The results of experiments shown in table 1 were conducted as follows: From a 10% stock solution of Grübler's dye in sterile distilled water, 1:100, 1:1000 and 1:10,000 dilutions were prepared with sterile water and distributed as follows in sterile test tubes:

1 c c brilliant green 1:100 + 8 c c glucose broth
1 c c brilliant green 1:1000 + 8 c c glucose broth
1 c c brilliant green 1:10,000 + 8 c c glucose broth
0.2 c c brilliant green 1:10,000 + 8.8 c c glucose broth
9 c c glucose broth (control)

To each tube was added 1 c c of a thoroughly emulsified 24-hour glucose broth culture of the diphtheria bacillus, the final dilutions of dye acting on the bacilli now being 1:1000, 1:10,000, 1:100,000 and 1:500,000. These mixtures were kept at 38 C. in a water bath and subcultured at intervals ranging from 5 minutes to 2 hours by planting several loopfuls on Loeffler's blood serum mediums and incubating for 48 hours.

The same tests were repeated with Hiss serum water glucose medium instead of glucose broth.

The controls always yielded luxuriant growths; the high diphthericidal activity of brilliant green in these tests is shown in table 1.

TABLE 1

THE DIPHTHERICIDAL ACTIVITY OF BRILLIANT GREEN (GRÜBLER)

Menstruum	Time Required for Complete Sterilization			
	1:1,000	1:10,000	1:100,000	1:500,000
Glucose broth..............	Less than 5 minutes	Less than 5 minutes	Less than 5 minutes	2 hours
Hiss serum water glucose..	....	10-20 minutes	More than 2 hours	More than 2 hours
Controls..................	....	Growth	Growth	Growth

The presence of serum in the Hiss medium evidently reduced the diphthericidal activity of the dye as compared with plain glucose broth; further evidence that protein reduces the bactericidal activity of brilliant green is shown in the results of the following experiments with citrated blood serum:

Five c c of human blood aspirated from a vein under aseptic conditions was added to 1 c c of a 10% solution of sodium citrate in salt solution; 1 c c of this citrated blood was mixed in small sterile test tubes with 1 c c of a 1:25,000 dilution of Grübler's dye and the mixture seeded with 0.1 c c of an emulsified 24-hour broth culture of the diphtheria bacillus. Similar mixtures were prepared with sterile unheated human serum and with sterile physiologic salt solution (control). These mixtures were kept at 38 C. in a water bath, and at intervals varying from 1 minute to 4 hours subcultures were made of each by transferring several loopfuls to Loeffler's medium.

Similar tests were conducted with 24-hour broth cultures of B. coli and Staph. aureus; the results are summarized in table 2.

TABLE 2

The Bactericidal Activity of 1:50,000 Solutions of Brilliant Green (Grübler)

Organisms	Time Required for Sterilization		
	In 50% Citrated Blood	In 50% Serum	In Salt Solution
B. diphtheriae	Not in 4 hours	4 hours	15 minutes
B. coli	Not in 4 hours	Not in 4 hours	Not in 4 hours
Staphylococcus aureus	Not in 4 hours	Not in 4 hours	15 minutes
Controls	Growth	Growth	Growth

While the dye in final dilutions of 1:50,000 proved completely bactericidal in 15 minutes for diphtheria bacilli and staphylococci in a menstruum of salt solution, sterilization was incomplete after 4 hours in the menstruum of blood and serum. The relatively low bactericidal activity of brilliant green for a gram-negative bacillus (B. coli) was well shown in these experiments.

Additional experiments were made with cultures of B. diphtheriae, B. typhosus, B. coli and Staph. aureus as follows: dilutions of Grübler's brilliant green varying from 1:1000 to 1:100,000 were prepared with sterile water and distributed in amounts varying from 0.1 to 0.8 c c in sterile test tubes; to each tube was added sufficient culture medium to bring the total volume to exactly 5 c c. Two mediums were employed, namely, Hiss serum water glucose and plain glucose broth; before adding the medium to the tubes containing the dye, each 100 c c was seeded with 1 c c of a 24-hour broth culture of the test micro-organism. The final dilutions of dye acting on this number of each micro-organism was readily calculated and the ease of manipulation rendered the technic particularly serviceable. Controls were included in which sterile salt solution replaced the dye.

All tubes were observed daily for a period of 5 days and then subcultured by transferring several loopfuls to Loeffler's medium. When the mediums remained clear and the subcultures proved sterile, the results were recorded as showing the bactericidal activity of brilliant green for the various micro-organisms; when the mediums remained clear over the period of 5 days but the subcultures proved positive, the results were recorded as showing the restraining, antiseptic or bacteriostatic activity of the dye.

Table 3 shows the highest dilutions of each of these two dyes proving bactericidal for the 4 micro-organisms in the 2 culture mediums; table 4 shows the highest dilutions proving bacteriostatic at the end of 5 days.

TABLE 3

THE BACTERICIDAL ACTIVITY OF BRILLIANT GREEN

Organisms	Grübler's Dye		Bayer's Dye	
	Hiss Serum Water	Glucose Broth	Hiss Serum Water	Glucose Broth
B. diphtheriae	1:125,000	1:625,000	1:125,000	1:500,000
B. typhosus	Not in 1:6,250	1:6,250	Not in 1:6,250	1:6,250
B. coli	1:6,250	1:6,250	Not in 1:6,250	1:6,250
Staphylococcus aureus	1:50,000	1:250,000	1:16,000	1:125,000
Controls	Growth	Growth	Growth	Growth

TABLE 4

THE BABTERIOSTATIC ACTIVITY OF BRILLIANT GREEN

Organisms	Grübler's Dye		Bayer's Dye	
	Hiss Serum Water	Glucose Broth	Hiss Serum Water	Glucose Broth
B. diphtheriae	1:160,000	1:820,000	1:160,000	1:820,000
B. typhosus	Not in 1:6,250	1:160,000	Not in 1:6,250	1:125,000
B. coli	1:8,200	1:12,000	Not in 1:6,250	1:8,200
Staphylococcus aureus	1:60,000	1:300,000	1:25,000	1:160,000

As shown in these tables, both dyes exhibited high degrees of bactericidal and bacteriostatic activity for the two gram-positive micro-organisms, namely, B. diphtheria and Staph. aureus and particularly in plain glucose broth; bactericidal and bacteriostatic activity for the cultures of typhoid and colon bacilli were very much less, both dyes being, however, more bactericidal and antiseptic for colon than for typhoid bacilli.

Additional experiments were made with Grübler's brilliant green in agar prepared and standardized after the method described by Krumwiede, Kohn, Kuttner and Schumm.[3] A stock 0.1% of the dye in sterile distilled water was used in amounts of 0.2, 0.3 and 0.5 c c added to 10 c c of the agar medium at 40 C.; the four test micro-organisms were cultivated in broth for 24 hours, thoroughly shaken and filtered through sterile paper. Each culture was employed in doses of 0.05, 0.1, 0.2 and 0.5 c c of varying dilutions placed in sterile petri dishes and poured with the brilliant green agar. Controls of each micro-organism were included, and always showed heavy growths. Each plate was examined after 24 and 48 hours' incubation. The results observed with 0.1 c c of 1:50 dilutions of B. diphtheria and Staph. aureus, 1:200 dilution of B. coli and 1:500 of B. typhosus, are given in table 5.

---

[3] Jour. Infect. Dis., 1918, 23, p. 275.

TABLE 5

THE BACTERICIDAL ACTIVITY OF BRILLIANT GREEN (GRÜBLER'S) IN AGAR PLATES AFTER 48 HOURS INCUBATION

Culture	0.1 c c of Dilution of Culture Plate for Each	Number of Colonies in 1 c c of Diluted Culture			
		With 0.2 c c of 0.1 % Stock Solution of Dye	With 0.3 c c of 0.1 % Stock Solution of Dye	With 0.5 c c of 0.1 % Stock Solution of Dye	No Dye
B. diphtheriae..........	1:50	None	None	None	Uncountable
B. typhosus............	1:500	Uncountable	Uncountable	Uncountable	Uncountable
B. coli..................	1:200	Uncountable	Uncountable	720	Uncountable
Staphylococcus aureus	1:50	None	None	None	Uncountable

As shown in table 5, brilliant green proved highly bactericidal for B. diphtheriae and Staph. aureus and much less bactericidal for B. typhosus and B. coli, but more for the latter than the former.

THE TREATMENT OF DIPHTHERIA CARRIERS WITH BRILLIANT GREEN *

A 1:250 solution of Grübler's brilliant green in distilled water was sprayed three times daily over the fauces of 22 persons and into the noses of 17 persons carrying diphtheria bacilli for 2 weeks or longer after recovery from faucial and nasal diphtheria; the solution was also instilled into the ears of 10 persons with chronic or subacute otitis media showing the presence of diphtheroid bacilli in the discharges. The dye proved nonirritating and nontoxic.

Our plan was to take cultures from each person in the morning, followed by three treatments during the day.

The results may be said to have been negative; frequently and indeed usually, treatment of throat, nose and ear carriers for from 1-3 days resulted in the disappearance of diphtheria and pseudodiphtheria bacilli, but never permanently. By keeping persons under observation for from 1-2 weeks and stopping the treatments with dye after the first negative culture, it was found that the cultures may remain negative for varying periods up to 10 days, followed by the reappearance of the bacilli in the cultures. Very probably brilliant green in the concentration employed exerted some diphthericidal effect, but its potency was reduced by the presence of protein material in the discharges, failure to penetrate the follicles of the tonsils and the difficulty of applying the solution in a thorough manner to the nasal chambers,

---

* We are indebted to Dr. R. L. Works for aid in this part of the investigation.

nasopharynx and pharynx of children. Since brilliant green also possesses a high degree of bactericidal activity for gram-positive cocci, such as staphylococci, streptococci and pneumococci, its use for local application in diphtheria and tonsillitis due to the cocci mentioned above, may prove of value and is certainly worthy of clinical trial.

CONCLUSIONS

Brilliant green has been found highly bactericidal and antiseptic for a virulent diphtheria bacillus in vitro; the presence of blood and serum in the medium reduced the bactericidal activity.

The dye was found also highly bactericidal and antiseptic for staphylococci.

Brilliant green possesses much less bactericidal and antiseptic activity for B. typhosus and B. coli, particularly for the former.

The use of brilliant green in the treatment of carriers of diphtheria and pseudodiphtheria bacilli usually resulted in the temporary disappearance of these bacilli from the noses, throats and ears of the treated persons; permanent ridding of these tissues of the bacilli, however, was not observed.

Owing to the high bactericidal activity of brilliant green for B. diphtheriae and such pathogenic cocci as staphylococci, streptococci and pneumococci, it is probable that the clinical use of a 1:250 solution of the drug in water may prove of value in the local treatment of infections caused by these organisms.